

09/882,509

=> d his

(FILE 'HOME' ENTERED AT 09:47:34 ON 11 MAY 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,  
LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004

L1           40597 S STREPTOKINASE?  
L2           362 S "S. EQUISIMILIS"  
L3           103 S L1 AND L2  
L4           6513995 S CLON? OR EXPRESS? OR RECOMBINANT  
L5           74 S L3 AND L4  
L6           29 DUP REM L5 (45 DUPLICATES REMOVED)  
L7           44795 S INCLUSION (A) BOD?  
L8           1 S L6 AND L7  
L9           42 S "LAMBDAPR"  
L10          0 S L2 AND L9  
L11          0 S L1 AND L9  
L12          0 S L9 (W) LAMBDAPL  
L13          0 S "LAMBDAPR-LAMBDAPL"  
L14          35 DUP REM L9 (7 DUPLICATES REMOVED)  
            E KUPPUSAMY M/AU  
L15          45 S E3-E6  
            E MOSUVAN M/AU  
            E MOSUVAN K/AU  
            E VELLIMEDU S/AU  
            E LAHIRI S/AU  
L16          1594 S E3  
            E KRISHNA E/AU  
L17          18 S E3-E7  
L18          1612 S L16 OR L17  
L19          0 S L1 AND L18

## Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID: SSSPTA1652MXM

**PASSWORD :**

TERMINAL (ENTER 1, 2, 3, OR ?):2

Enter NEWS followed by the item number or name to see news on that specific topic.

All use of STN is subject to the provisions of the STN Customer agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

FILE 'HOME' ENTERED AT 09:47:34 ON 11 MAY 2004

=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci  
COST IN U.S. DOLLARS SINCE FILE TOTAL  
ENTDV SESSION

FULL ESTIMATED COST 0.21 0.21

FILE 'MEDLINE' ENTERED AT 09:47:55 ON 11 MAY 2004

FILE 'EMBASE' ENTERED AT 09:47:55 ON 11 MAY 2004  
COPYRIGHT (C) 2004 Elsevier Inc. All rights reserved.

FILE 'BIOSIS' ENTERED AT 09:47:55 ON 11 MAY 2004  
COPYRIGHT (C) 2004 BIOLOGICAL ABSTRACTS INC. (R)

FILE 'BIOTECHDS' ENTERED AT 09:47:55 ON 11 MAY 2004  
COPYRIGHT (C) 2004 THOMSON DERWENT AND INSTITUTE FOR SCIENTIFIC INFORMATION

FILE 'SCISEARCH' ENTERED AT 09:47:55 ON 11 MAY 2004  
COPYRIGHT 2004 THOMSON ISI

FILE 'HCAPLUS' ENTERED AT 09:47:55 ON 11 MAY 2004  
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.  
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.  
COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'NTIS' ENTERED AT 09:47:55 ON 11 MAY 2004  
Compiled and distributed by the NTIS, U.S. Department of Commerce.  
It contains copyrighted material.  
All rights reserved. (2004)

FILE 'LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004  
COPYRIGHT (C) 2004 Cambridge Scientific Abstracts (CSA)

=> s streptokinase2  
2 IS NOT A RECOGNIZED COMMAND  
The previous command name entered was not recognized by the system.  
For a list of commands available to you in the current file, enter  
"HELP COMMANDS" at an arrow prompt (>).

=> s streptokinase?  
L1 40597 STREPTOKINASE?  
  
=> s "S. equisimilis"  
L2 362 "S. EQUISIMILIS"  
  
=> s l1 and l2  
L3 103 L1 AND L2  
  
=> s clon? or express? or recombinant  
5 FILES SEARCHED...  
L4 6513995 CLON? OR EXPRESS? OR RECOMBINANT

=> s l3 and l4  
L5 74 L3 AND L4

=> dup rem 15  
PROCESSING COMPLETED FOR L5  
L6 29 DUP REM L5 (45 DUPLICATES REMOVED)

=> d 1-29 ibib ab

L6 ANSWER 1 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN DUPLICATE 1  
ACCESSION NUMBER: 2003404343 EMBASE  
TITLE: Expression of streptodornase by use of  
streptokinase promoter in Streptococcus equisimilis  
H46A.

AUTHOR: Sohn H.-J.; Chin J.; Kim I.-C.; Bai S.; Lee H.B.  
CORPORATE SOURCE: H.B. Lee, Department of Biological Sciences, Chonnam  
National University, Gwangju 500-757, Korea, Republic of.  
blaise@chonnam.chonnam.ac.kr  
SOURCE: Korean Journal of Microbiology and Biotechnology, (2003)  
31/3 (307-310).  
Refs: 18  
ISSN: 1598-642X CODEN: HMHAAS  
COUNTRY: Korea, Republic of  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: Korean  
SUMMARY LANGUAGE: English

AB A gene encoding streptodornase(sdc) from *Streptococcus equisimilis* H46A was expressed in *S. equisimilis* H46A sdc(-) under the control of the **streptokinase** gene promoter. Secretion of the streptodornase was directed by the signal sequences of **streptokinase** or streptodornase. The expressed streptodornase activity from *S. equisimilis* H46A sdc(-) transformant with **streptokinase** promoter - streptodornase coding sequence fusion vector was 2.3 fold higher than that from wild type. Construct of signal sequence region replaced by **streptokinase** ones was similarly expressed as a wild type. But constructs of skc or lrp core regions of **streptokinase** promoter streptodornase fusion were similarly expressed as in sdc(-) mutant. In conclusion, improved expression of streptodornase by use of **streptokinase** promoter required the full length of promoter.

L6 ANSWER 2 OF 29 MEDLINE on STN DUPLICATE 2  
ACCESSION NUMBER: 2000038313 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10569766  
TITLE: Cloning, expression, sequence analysis, and characterization of **streptokinases** secreted by porcine and equine isolates of *Streptococcus equisimilis*.  
AUTHOR: Caballero A R; Lottenberg R; Johnston K H  
CORPORATE SOURCE: Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, New Orleans, Louisiana 70112, USA.  
CONTRACT NUMBER: R01DK45014 (NIDDK)  
SOURCE: Infection and immunity, (1999 Dec) 67 (12) 6478-86.  
Journal code: 0246127. ISSN: 0019-9567.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AF104300; GENBANK-AF104301  
ENTRY MONTH: 199912  
ENTRY DATE: Entered STN: 20000113  
Last Updated on STN: 20000113  
Entered Medline: 19991220

AB **Streptokinases** secreted by nonhuman isolates of group C streptococci (*Streptococcus equi*, *S. equisimilis*, and *S. zooepidemicus*) have been shown to bind to different mammalian plasminogens but exhibit preferential plasminogen activity. The **streptokinase** genes from *S. equisimilis* strains which activated either equine or porcine plasminogen were cloned, sequenced, and expressed in *Escherichia coli*. The **streptokinase** secreted by the equine isolate had little similarity to any known **streptokinases** secreted by either human or porcine isolates. The **streptokinase** secreted by the porcine isolate had limited structural and functional similarities to **streptokinases** secreted by human isolates. Plasminogen activation

studies with immobilized (His) (6)-tagged recombinant streptokinases indicated that these recombinant streptokinases interacted with plasminogen in a manner similar to that observed when streptokinase and plasminogen interact in the fluid phase. Analysis of the cleavage products of the streptokinase-plasminogen interaction indicated that human, equine, and porcine plasminogens were all cleaved at the same highly conserved site. The site at which streptokinase was cleaved to form altered streptokinase (Sk\*) was also determined. This study confirmed not only the presence of streptokinases in nonhuman *S. equisimilis* isolates but also that these proteins belong to a family of plasminogen activators more diverse than previously thought.

L6 ANSWER 3 OF 29 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
DUPLICATE 3  
ACCESSION NUMBER: 2000:96119 BIOSIS  
DOCUMENT NUMBER: PREV200000096119  
TITLE: Two streptokinase genes are expressed with different solubility in Escherichia coli W3110.  
AUTHOR(S): Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens, Isis  
CORPORATE SOURCE: Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba  
SOURCE: Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.  
CODEN: BILED3. ISSN: 0141-5492.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 15 Mar 2000  
Last Updated on STN: 3 Jan 2002

AB The streptokinase (SK) gene from *S. equisimilis* H46A (ATCC 12449) was cloned in *E. coli* W3110 under the control of the tryptophan promoter. The recombinant SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from *S. equisimilis* (ATCC 9542) which had been cloned in *E. coli* W3110 by using similar expression vector and cell growth conditions, and occurred in the form of inclusion bodies.

L6 ANSWER 4 OF 29 MEDLINE on STN DUPLICATE 4  
ACCESSION NUMBER: 1999150235 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10024545  
TITLE: Purification and cloning of a streptokinase from *Streptococcus uberis*.  
AUTHOR: Johnsen L B; Poulsen K; Kilian M; Petersen T E  
CORPORATE SOURCE: Protein Chemistry Laboratory, Department of Molecular and Structural Biology, University of Aarhus, DK-8000 Aarhus C, Denmark.  
SOURCE: Infection and immunity, (1999 Mar) 67 (3) 1072-8.  
Journal code: 0246127. ISSN: 0019-9567.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AJ131604; GENBANK-AJ131605; GENBANK-AJ131631  
ENTRY MONTH: 199903  
ENTRY DATE: Entered STN: 19990326  
Last Updated on STN: 19990326  
Entered Medline: 19990312  
AB A bovine plasminogen activator was purified from the culture supernatant

of the bovine pathogen *Streptococcus uberis* NCTC 3858. After the final reverse-phase high-performance liquid chromatography step a single protein with a molecular mass of 32 kDa was detected in the active fraction. A partial peptide map was established, and degenerate primers were designed and used for amplification of fragments of the gene encoding the activator. Inverse PCR was subsequently used for obtaining the full-length gene. The *S. uberis* plasminogen activator gene (*skc*) encodes a protein consisting of 286 amino acids including a signal peptide of 25 amino acids. In an amino acid sequence comparison the **cloned** activator showed an identity of approximately 26% to the **streptokinases** isolated from *Streptococcus equisimilis* and *Streptococcus pyogenes*. Interestingly, the activator from *S. uberis* was found to lack the C-terminal domain possessed by the **streptokinase** from *S. equisimilis*. This is apparently a general feature of the **streptokinases** of this species; biochemical and genetic analysis of 10 additional strains of *S. uberis* revealed that 9 of these were highly similar to strain NCTC 3858. Sequencing of the *skc* gene from three of these strains indicated that the amino acid sequence of the protein is highly conserved within the species.

L6 ANSWER 5 OF 29 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
ACCESSION NUMBER: 1999-03949 BIOTECHDS

TITLE: Purification and **cloning** of a **streptokinase**  
from *Streptococcus uberis*;  
cattle plasminogen-activator purification and  
characterization

AUTHOR: Johnson L B; Poulsen K; Kilian M; \*Petersen T E

CORPORATE SOURCE: Univ.Aarhus

LOCATION: Protein Chemistry Laboratory, Gustav Wieds Vej 10C, DK-8000  
Aarhus C, Denmark.

SOURCE: Email: tep@mbio.aau.dk  
*Infect.Immun.*; (1999) 67, 3, 1072-78  
CODEN: INFIBR  
ISSN: 0019-9567

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB A cattle plasminogen-activator was purified from the culture supernatant of *Streptococcus uberis* NTCTC 3858. After the final reverse-phase HPLC step, a single protein with a mol.weight of 32,000 was detected in the active fraction. A partial peptide map was established, and degenerate DNA primers were designed and used for amplification of fragments of the gene encoding the activator. Inverse polymerase chain reaction was used for obtaining the full-length gene. The *S. uberis* plasminogen-activator gene (*skc*) encodes a protein consisting of 286 amino acids including a signal peptide of 25 amino acids. In a protein sequence comparison, the **cloned** activator showed an identity of approximately 26% to the **streptokinases** isolated from *Streptococcus equisimilis* and *Streptococcus pyogenes*. The activator from *S. uberis* lacked the C-terminal domain possessed by the **streptokinase** from *S. equisimilis*. This is apparently a general feature of the **streptokinases** of this species. Sequencing of the *skc* gene from 3 of these strains indicated that the protein sequence of the protein is highly conserved within the species. (32 ref)

L6 ANSWER 6 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN  
ACCESSION NUMBER: 97:555242 SCISEARCH

THE GENUINE ARTICLE: XL484

TITLE: The LppC gene of *Streptococcus equisimilis* encodes a lipoprotein that is homologous to the e(P4) outer membrane protein from *Haemophilus influenzae*

AUTHOR: Gase K; Liu G W; Bruckmann A; Steiner K; Ozegowski J;  
Malke H (Reprint)

CORPORATE SOURCE: UNIV JENA, INST MOL BIOL, WINZERLAER STR 10, D-07745 JENA,  
GERMANY (Reprint); UNIV JENA, INST MOL BIOL, D-07745 JENA,

COUNTRY OF AUTHOR: GERMANY  
GERMANY  
SOURCE: MEDICAL MICROBIOLOGY AND IMMUNOLOGY, (JUN 1997) Vol. 186,  
No. 1, pp. 63-73.  
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY  
10010.  
ISSN: 0300-8584.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 39

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We report the cloning, sequencing, and analysis of a novel chromosomal gene of *Streptococcus equisimilis* strain H46A that codes for a membrane lipoprotein, designated LppC. The lppC gene is located 3' adjacent to, and co-oriented with, the unrelated gapC gene that encodes the previously characterized glyceraldehyde-3-phosphate dehydrogenase. Sequencing of lppC revealed an 855-bp open reading frame that predicted a 32.4-kDa polypeptide possessing a potential lipoprotein signal sequence and modification site (VTGC). Signal sequence processing of LppC synthesized in the homologous host or expressed from plasmid pLPP2 in *Escherichia coli* was sensitive to globomycin, a selective inhibitor of lipoprotein-specific signal peptidase II. Subcellular localization of LppC using polyclonal antibodies raised to the hexahistidyl-tagged protein proved LppC to be tightly associated with the cytoplasmic membrane of *S. equisimilis* and with the outer membrane of *E. coli* JM109 (pLPP2). Southern, Northern and Western analyses indicated that Ipl, was conserved in *S. pyogenes*, and transcribed independently of gap as monocistronic 0.9-kb mRNA from a sigma(70)-like consensus promoter. Database searches found homology of LppC to the hel gene-encoded outer membrane protein e (P4) from *Haemophilus influenzae* to which it exhibits 58% sequence similarity. However, unlike the hel gene, lppC was unable to complement hemA mutants of *E. coli* for growth on hemin as sole porphyrin source in aerobic conditions. Furthermore, neither the wild type nor an lppC insertion mutant of *S. equisimilis* could grow on hemin in iron-limited medium. These results, together with findings indicating that *S. equisimilis* H46A had no absolute requirement for iron, led us to conclude that lppC, in contrast to hel, is not involved in hemin utilization and has yet to be assigned a function.

L6 ANSWER 7 OF 29 LIFESCI COPYRIGHT 2004 CSA on STN  
ACCESSION NUMBER: 96:44932 LIFESCI  
TITLE: Functional analysis of a relA/spoT gene homolog from  
*Streptococcus equisimilis*  
AUTHOR: Mechold, U.; Cashel, M.; Steiner, K.; Gentry, D.; Malke, H.  
CORPORATE SOURCE: Inst. Molecular Biol., Jena Univ., Winzerlaer Str. 10,  
D-07745 Jena, Germany  
SOURCE: J. BACTERIOL., (1996) vol. 178, no. 5, pp. 1404-1411.  
ISSN: 0021-9193.  
DOCUMENT TYPE: Journal  
FILE SEGMENT: J; G  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB We examined the functional attributes of a gene encountered by sequencing the streptokinase gene region of *Streptococcus equisimilis* H46A. This gene, originally called rel, here termed rel sub( )s. *equisimilis*, is homologous to two related *Escherichia coli* genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of *E. coli* mutants led us to deduce that the highly expressed rel sub( )s . *equisimilis* gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA

gene. We verified that the Rel sub( )*S. equisimilis* protein, purified from an *E. coli* relA spot double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel sub( )*S. equisimilis* protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel sub( )*S. equisimilis* protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel sub( )*S. equisimilis* gene function in *E. coli* as being similar to that of the native spoT gene, disruptions of rel sub( )*S. equisimilis* abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L6 ANSWER 8 OF 29 MEDLINE on STN DUPLICATE 5  
 ACCESSION NUMBER: 96200111 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8631718  
 TITLE: Functional analysis of a relA/spoT gene homolog from *Streptococcus equisimilis*.  
 AUTHOR: Mechold U; Cashel M; Steiner K; Gentry D; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Journal of bacteriology, (1996 Mar) 178 (5) 1401-11.  
 Journal code: 2985120R. ISSN: 0021-9193.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199607  
 ENTRY DATE: Entered STN: 19960715  
 Last Updated on STN: 19970203  
 Entered Medline: 19960703

AB We examined the functional attributes of a gene encountered by sequencing the **streptokinase** gene region of *Streptococcus equisimilis* H46A. This gene, originally called *rel*, here termed *rels*. *equisimilis*, is homologous to two related *Escherichia coli* genes, *spoT* and *relA*, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of *E. coli* mutants led us to deduce that the highly expressed *rel S. equisimilis* gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the *spoT* gene, with a net effect favoring degradation and no complementation of the absence of the *relA* gene. We verified that the Rel *S. equisimilis* protein, purified from an *E. coli* relA spot double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel *S. equisimilis* protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel *S. equisimilis* protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel *S. equisimilis* gene function in *E. coli* as being similar to that of the native spoT gene, disruptions of rel *S. equisimilis* abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L6 ANSWER 9 OF 29 MEDLINE on STN DUPLICATE 6  
 ACCESSION NUMBER: 96396845 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8803948  
 TITLE: Structural dissection and functional analysis of the

complex promoter of the **streptokinase** gene from  
 Streptococcus equisimilis H46A.  
 AUTHOR: Grafe S; Ellinger T; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Medical microbiology and immunology, (1996 May) 185 (1)  
 11-7.  
 Journal code: 0314524. ISSN: 0300-8584.  
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199701  
 ENTRY DATE: Entered STN: 19970219  
 Last Updated on STN: 19970219  
 Entered Medline: 19970131

AB The overlapping tandem promoters of the **streptokinase** gene, P1 and P2, identified previously by S1 nuclease transcript mapping were functionally dissected by mutagenesis of their -10 regions and fused transcriptionally with or without the 202-bp upstream region (USR) to the luciferase reporter gene (luc) from Photinus pyralis to analyze the contribution of the different sequence elements to promoter activity in Escherichia coli and the homologous Streptococcus equisimilis strain H46A. In E. coli, virtually the entire promoter activity derived from the upstream promoter P1. In **S. equisimilis**, luc expression increased in the following order of the involved sequence elements: P2 approximately equal to P2 + USR < P1 < P1 + P2 < P1 + USR < P1 + P2 + USR. This shows that (1) in the homologous system, P1 and P2 alone are extremely weak, (2) in the USR-less arrangement, only the combined core promoters have substantial activity, and (3) the USR stimulates only P1 and the combination of P1 + P2. Thus, the tandem promoters presumably function by mutual contributory action and their full activity strongly depends on the AT-rich and statically bent upstream region. The distinctive feature determining the strength of P1 in both hosts appears to be its extended -10 region which matches the consensus TRTGN established for strong **S. pneumoniae** and **Bacillus subtilis** promoters.

L6 ANSWER 10 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN  
 ACCESSION NUMBER: 2000:433656 HCAPLUS  
 DOCUMENT NUMBER: 133:27355  
 TITLE: Cloning and expression of Streptococcus H46 **streptokinase** gene  
 INVENTOR(S): Cho, Jung-Myong; Park, Yong-U.  
 PATENT ASSIGNEE(S): LG Chemical Co., Ltd., S. Korea  
 SOURCE: Repub. Korea, No pp. given  
 CODEN: KRXXFC  
 DOCUMENT TYPE: Patent  
 LANGUAGE: Korean  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
KR 9512901	B1	19951023	KR 1992-17406	19920924
PRIORITY APPLN. INFO.:			KR 1992-17406	19920924

AB The cloning of **streptokinase** gene of Streptococcus H46 consists of PCR with primers and cloning the gene into the PstI-NdeI site of plasmid ptrp322H-HGH (KFCC 10067) to get ptrpH-SK (ATCC 68884). The DNA sequence of Streptococcus H46 **streptokinase** has 92.2-98.8% homol. to SKC, SKG, and SKA. Streptococcus H46 is also designated **S. equisimilis** ATCC 35556.

L6 ANSWER 11 OF 29 MEDLINE on STN DUPLICATE 7  
 ACCESSION NUMBER: 95342169 MEDLINE

DOCUMENT NUMBER: PubMed ID: 7616967  
TITLE: Complex transcriptional control of the  
**streptokinase** gene of *Streptococcus equisimilis*  
H46A.  
AUTHOR: Gase K; Ellinger T; Malke H  
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
SOURCE: Molecular & general genetics : MGG, (1995 Jun 25) 247 (6)  
749-58.  
Journal code: 0125036. ISSN: 0026-8925.  
PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199508  
ENTRY DATE: Entered STN: 19950905  
Last Updated on STN: 19950905  
Entered Medline: 19950822

AB On the *Streptococcus equisimilis* H46A chromosome, the divergent coding sequences of the genes for the plasminogen activator **streptokinase** (*skc*) and a leucine-rich protein (*lrp*), the function of which is unknown, are separated by a 328 bp intrinsically bent DNA region rich in AT tracts. To begin to understand the **expression** control of these two genes, we mapped their transcriptional initiation sites by S1 nuclease analysis and studied the influence of the bent intergenic region on promoter strength, using promoter-reporter gene fusions of *skc'* and *lrp'* to '*lacZ*' from *Escherichia coli*. The major transcriptional start sites, in both *S. equisimilis* and *E. coli*, mapped 22 bases upstream of the ATG start site of *lrp* (G), and 24 and 32 bases upstream of the translational initiation codon of *skc* (A and G, respectively), indicating the existence of two overlapping canonical *skc* promoters arranged in tandem on opposite faces of the helix. The reporter gene fusions were **cloned** in *E. coli* on a vector containing a 1.1 kb fragment of the *S. equisimilis* *dexB* gene, thus allowing promoter strength to be measured in multiple plasmid-form copies in the heterologous host and in single-copy genomic form following integration into the *skc* region of the homologous host. In *S. equisimilis*, *skc'*-'*lacZ* was **expressed** about 200-fold more strongly than the corresponding *lrp'*-'*lacZ* fusion. In contrast, in *E. coli*, the corresponding levels of **expression** differed by only about 11-fold. Deletion of the 202 bp bent region upstream of the *skc* and *lrp* core promoters caused a 13-fold decrease in *skc* promoter activity in *S. equisimilis* but did not alter *lrp* promoter strength in this host. In contrast, when studied in *E. coli*, this deletion did not alter the strength of the *skc*-double promoter and even increased by 2.4- to 3-fold the activity of the *lrp* promoter. This comparative promoter analysis shows that *skc* has a complex promoter structure, the activity of which in the homologous genomic environment specifically depends on sequences upstream of the two core promoters. Thus, the *skc* promoter structure resembles that of an array of promoters involved in a transcriptional switch; however, the nature of the potential switch factor(s) remains unknown.

L6 ANSWER 12 OF 29 MEDLINE on STN DUPLICATE 8  
ACCESSION NUMBER: 95157528 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 7531815  
TITLE: Transcription termination of the **streptokinase** gene of *Streptococcus equisimilis* H46A: bidirectionality and efficiency in homologous and heterologous hosts.  
AUTHOR: Steiner K; Malke H  
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
SOURCE: Molecular & general genetics : MGG, (1995 Feb 6) 246 (3)  
374-80.  
Journal code: 0125036. ISSN: 0026-8925.  
PUB. COUNTRY: GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199503

ENTRY DATE: Entered STN: 19950322

Last Updated on STN: 19960129

Entered Medline: 19950316

AB In *Streptococcus equisimilis* H46A, a hypersymmetrical transcription terminator with bidirectional activity was localized between the translational termination codons of the **streptokinase** gene, skc, and the rel-orf1 genes. These two transcription units are oriented towards each other, and under normal conditions the skc mRNA level exceeds that of the rel-orf1 genes by a factor of at least 1000. Reporter vectors based on the promoterless cat gene were constructed by transcriptional fusion of skc to cat, such that the region between the two genes contained the terminator in skc orientation or in rel-orf1 orientation. Additionally, skc and cat were fused directly, with deletion of the terminator. The reporter vectors were designed to be capable of being studied either as multicopy plasmids in *Escherichia coli* or in single copy following integration, via skc, into the *S. equisimilis* chromosome. Chloramphenicol acetyl transferase (CAT) activity assays in conjunction with determination of chloramphenicol resistance levels and Northern hybridization analysis showed that the terminator is active in either host and orientation. However, termination efficiency was host dependent, with high terminator strength being observed in the homologous streptococcal background and appreciable readthrough occurring in *E. coli*. The extent of transcriptional readthrough was dependent upon terminator orientation, with termination being more efficient in rel-orf1 polarity. The results suggest that, in *S. equisimilis*, transcription of both skc and rel-orf1 is efficiently terminated by a common signal, and that these genes are largely protected from convergent transcription, which otherwise would seem to be particularly detrimental to the weakly expressed rel-orf1 genes.

L6 ANSWER 13 OF 29 MEDLINE on STN

DUPPLICATE 9

ACCESSION NUMBER: 96154934 MEDLINE

DOCUMENT NUMBER: PubMed ID: 8577315

TITLE: Conservation of the organization of the **streptokinase** gene region among pathogenic streptococci.

AUTHOR: Frank C; Steiner K; Malke H

CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.

SOURCE: Medical microbiology and immunology, (1995 Oct) 184 (3) 139-46.

Journal code: 0314524. ISSN: 0300-8584.

GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-X72832

ENTRY MONTH: 199603

ENTRY DATE: Entered STN: 19960321

Last Updated on STN: 19960321

Entered Medline: 19960313

AB Using ten gene-specific probes from the cloned and sequenced **streptokinase** gene (skc) region (8,931 bp) of *Streptococcus equisimilis* H46A, a human serogroup C strain, the conservation of these genes and their linkage relationships were studied by Southern hybridization in pathogenic streptococci differing taxonomically, serologically, in regard to their host range, and in the class of plasminogen activator produced. The results indicate that in *S. pyogenes* (strains A374, NZ131 and SF130/13) and a human group G strain (G19,908) both gene content and gene order as determined for H46A (dexB-abc-lrp-skc-orf1-rel) are preserved. The same is true of an equine

**S. equisimilis** isolate (87-542-W), the **streptokinase** gene of which has been shown to hybridize detectably with skc, a result at variance with that obtained previously by others. In contrast, the chromosomal DNA of three *S. uberis* strains (0140J, C198, C216) of bovine origin, two of which produced a plasminogen activator different from **streptokinase**, hybridized only with dexB-, abc- and rel-specific probes, and the homologues of these genes appeared to lie close to each other. The maintenance of the organization of the **streptokinase** gene region in strains differing in overall chromosomal character suggests that this gene arrangement is of selective advantage.

L6 ANSWER 14 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1994:186995 HCAPLUS

DOCUMENT NUMBER: 120:186995

TITLE: Inactivation of the **streptokinase** gene prevents *Streptococcus equisimilis* H46A from acquiring cell-associated plasmin activity in the presence of plasminogen

AUTHOR(S): Malke, Horst; Mechold, Undine; Gase, Klaus; Gerlach, Dieter

CORPORATE SOURCE: Inst. Mol. Biol., Jena Univ., Jena, D-07745, Germany

SOURCE: FEMS Microbiology Letters (1994), 116(1), 107-12

CODEN: FMLED7; ISSN: 0378-1097

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The **streptokinase** gene of *S. equisimilis* H46 was inactivated by plasmid insertion mutagenesis to study the relation between elaboration of **streptokinase** and acquisition of cell-associated plasmin activity after incubation of wild-type and mutant cells in media containing plasminogen or plasmin. H46A binds both the zymogen and active enzyme, generates surface-associated plasmin activity in the presence of plasminogen when producing **streptokinase**, and expresses its plasmin(ogen) receptor(s) independently of a functional **streptokinase** gene. At least part of the plasmin(ogen) binding capacity may be due to the glyceraldehyde-3-phosphate dehydrogenase type of receptor mol., as judged by the detection of the corresponding gene.

L6 ANSWER 15 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1992:441935 HCAPLUS

DOCUMENT NUMBER: 117:41935

TITLE: Cloning and expression of **streptokinase** gene of C-type *Streptococcus equisimilis*

PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba

SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 04030794	A2	19920203	JP 1990-201600	19900731
JP 3127298	B2	20010122		
EP 489201	A1	19920610	EP 1990-201930	19900717
EP 489201	B1	19951115		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 130369	E	19951215	AT 1990-201930	19900717
ES 2081909	T3	19960316	ES 1990-201930	19900717
US 5296366	A	19940322	US 1991-703778	19910522

AU 644657 B2 19931216 AU 1991-78101 19910531  
RU 2107726 C1 19980327 RU 1991-5001280 19910717  
PRIORITY APPLN. INFO.: CU 1990-90 A 19900523  
SU 1991-5001280 A 19910717

AB The **streptokinase** (I) gene SKC-2 ,with/without signal sequence, is cloned from C-type *S. equisimilis* ATCC-9542 by the polymerase chain reaction method and expressed in Escherichia coli and yeast for com. manufacture of I. Genomic DNA of the C-type *S. equisimilis* was isolated by the standard method and amplified with primers derived from the nucleotide sequence of SKC to get I gene with/without signal sequence. Expression of the I gene in E. coli and Pichia pastoris MP-36 mutant were shown. The production of I with these microorganisms were ≥350 mg/L and ≥1.2 g/L, resp.

L6 ANSWER 16 OF 29 HCPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1992:646505 HCPLUS  
DOCUMENT NUMBER: 117:246505  
TITLE: **Streptokinase** mutation affecting skc expression in homologous and heterologous hosts  
AUTHOR(S): Mechold, U.; Muller, J.; Malke, H.  
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Jena, D-6900, Germany  
SOURCE: Zentralblatt fuer Bakteriologie, Supplement (1992), 22 (New Perspect. Streptococci Streptococcal Infect.), 336-8  
DOCUMENT TYPE: CODEN: ZBASE2; ISSN: 0941-018X  
LANGUAGE: English

AB Mutations affecting the level of **streptokinase** gene skc expression and/or secretion in homologous and heterologous hosts are phys. characterized. The principal classes of mutations produced included skc deletions, IS element insertions, and skc duplications. The deletion events, represented by mutations Δ(skc)-247 and Δ(skc)-305 present in plasmids pMM247 and pMM305, resp., removed a tetrapeptide (F10-L13 or L12-A15) from the hydrophobic core of the Skc signal sequence. These mutations, reduced the size, hydrophobicity and predicted alpha-helicity of the central region of the signal sequence. The corresponding plasmids, upon transformation into E. coli and *P. mirabilis* L-forms, substantially increased the level of Skc expression in either host. In E. coli, they also facilitated the export of mature Skc into the culture medium. In the gram-pos. hosts, skc expression was less dramatically affected; however, the proportion of Skc activity found in the culture medium was significantly decreased when compared to the extracellular activity resulting from wild type skc. IS1 insertion did not alter the primary structure of the promoter but displaced in upward direction, by 768 bp, a static DNA bending locus having its center some 140 bp upstream of the -35 region in wild type DNA. When studied with plasmid pMM697, this insertion event resulted in severely decreased Skc expression in all hosts but, expectedly, did not affect Skc secretability. Gene skc duplication in the chromosome of the homologous producer strain, *S. equisimilis* H46A, was achieved by a single crossover event between the chromosomes and an integrateable Skc plasmid, pSM752, in the region of shared homol. As judged by Southern hybridization, cells transiently supporting the replication of pSM752 gave rise to a stable erythromycin-resistant clone designated H46SM which was plasmid-free and produced Skc at levels approx. twice as high as the wild type.

L6 ANSWER 17 OF 29 MEDLINE on STN DUPLICATE 10  
ACCESSION NUMBER: 92039051 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 1937032  
TITLE: Isolation, sequence and expression in Escherichia

coli, *Bacillus subtilis* and *Lactococcus lactis* of the DNase (streptodornase)-encoding gene from *Streptococcus equisimilis* H46A.  
AUTHOR: Wolinowska R; Ceglowski P; Kok J; Venema G  
CORPORATE SOURCE: Department of Pharmaceutical Microbiology, Medical Academy, Warsaw, Poland.  
SOURCE: Gene, (1991 Sep 30) 106 (1) 115-9.  
Journal code: 7706761. ISSN: 0378-1119.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-M59725; GENBANK-M59726; GENBANK-M59727;  
GENBANK-M59728; GENBANK-M63990; GENBANK-S61507;  
GENBANK-S63856; GENBANK-S63863; GENBANK-S65020;  
GENBANK-S65060; GENBANK-X17241  
ENTRY MONTH: 199112  
ENTRY DATE: Entered STN: 19920124  
Last Updated on STN: 19920124  
Entered Medline: 19911223

AB A partial library of BclI-generated chromosomal DNA fragments from *Streptococcus equisimilis* H64A (Lancefield Group C) was constructed in *Escherichia coli*. Clones displaying either **streptokinase** or deoxyribonuclease (streptodornase; SDC) activities were isolated. The gene (sdc) expressing the SDC activity was allocated on the 1.1-kb AccI DNA subfragment. Sequence analysis of this DNA fragment revealed the presence of one open reading frame, which could encode a protein of 36.8 kDa. The N-terminal portion of the deduced protein exhibited features characteristic of prokaryotic signal peptides. The sdc gene was expressed in *E. coli*, *Bacillus subtilis* and *Lactococcus lactis*. As observed for *S. equisimilis*, in the heterologous Gram + hosts, at least part of the SDC protein was secreted into the medium.

L6 ANSWER 18 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN  
ACCESSION NUMBER: 91:636602 SCISEARCH  
THE GENUINE ARTICLE: GQ068  
TITLE: ISOLATION, SEQUENCE AND EXPRESSION IN  
ESCHERICHIA-COLI, BACILLUS-SUBTILIS AND LACTOCOCCUS-LACTIS  
OF THE DNASE (STREPTODORNASE)-ENCODING GENE FROM  
STREPTOCOCCUS-EQUISIMILIS H46A  
AUTHOR: WOLINOWSKA R; CEGLOWSKI P (Reprint); KOK J; VENEMA G  
CORPORATE SOURCE: MED ACAD WARSAW, DEPT PHARMACEUT MICROBIOL, OCZKI 3,  
PL-02007 WARSAW, POLAND; UNIV GRONINGEN, INST GENET, 9700  
AB GRONINGEN, NETHERLANDS  
COUNTRY OF AUTHOR: POLAND; NETHERLANDS  
SOURCE: GENE, (1991) Vol. 106, No. 1, pp. 115-119.  
DOCUMENT TYPE: Note; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 32

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A partial library of BclI-generated chromosomal DNA fragments from *Streptococcus equisimilis* H64A (Lancefield Group C) was constructed in *Escherichia coli*. Clones displaying either **streptokinase** or deoxyribonuclease (streptodornase; SDC) activities were isolated. The gene (sdc) expressing the SDC activity was allocated on the 1.1-kb AccI DNA subfragment. Sequence analysis of this DNA fragment revealed the presence of one open reading frame, which could encode a protein of 36.8 kDa. The N-terminal portion of the deduced protein exhibited features characteristic of prokaryotic signal peptides. The sdc gene was expressed in *E. coli*, *Bacillus subtilis* and *Lactococcus lactis*. As observed for *S. equisimilis*, in the heterologous Gram+ hosts, at least part of the SDC protein was secreted

into the medium.

L6 ANSWER 19 OF 29 HCPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1991:179756 HCPLUS  
DOCUMENT NUMBER: 114:179756  
TITLE: Manufacture of serotype c **streptokinase** with  
recombinant *Streptococcus equisimilis*  
INVENTOR(S): Mueller, Joerg; Malke, Horst  
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Ger. Dem. Rep.  
SOURCE: Ger. (East), 12 pp.  
CODEN: GEXXA8  
DOCUMENT TYPE: Patent  
LANGUAGE: German  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 284898	A5	19901128	DD 1989-332866	19890921
PRIORITY APPLN. INFO.:			DD 1989-332866	19890921

AB Serotype c **streptokinase** is manufactured by *S. equisimilis* transformed with a plasmid containing the *S. equisimilis* skc gene and a selectable marker, preferably the erythromycin resistance gene. The plasmid becomes incorporated into the microbial genome by recombination to double the skc gene copy number to two. Submerged cultivation of the transformant results in the enzyme being secreted into the medium in quantities apprx.2-fold greater than those secreted by the wild-type strain.

L6 ANSWER 20 OF 29 HCPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1990:625400 HCPLUS  
DOCUMENT NUMBER: 113:225400  
TITLE: Duplication of the **streptokinase** gene in the chromosome of *Streptococcus equisimilis* H46A  
AUTHOR(S): Mueller, Joerg; Malke, Horst  
CORPORATE SOURCE: Acad. Sci. GDR, Cent. Inst. Microbiol. Exp. Ther., Jena, DDR-6900, Ger. Dem. Rep.  
SOURCE: FEMS Microbiology Letters (1990), 72(1-2), 75-8  
CODEN: FMLED7; ISSN: 0378-1097  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The erythromycin resistance plasmid pSM752 carrying the cloned **streptokinase** gene, skc, was introduced by protoplast transformation into *S. equisimilis* H46A from which skc was originally cloned. Cells transiently supporting the replication of pSM752 gave rise to an erythromycin-resistant clone designated H46SM which was plasmid free and produced **streptokinase** at levels approx. twice as high as the wild type. Southern hybridization of total cell DNA with an skc-containing probe provided evidence for the duplication of the skc gene in the H46SM chromosome. The results, which have some bearing on industrial **streptokinase** production, can be best explained by a single cross-over event between the chromosome and the plasmid in the region of shared homol. leading to the integration of pSM752 in a Campbell-like manner.

L6 ANSWER 21 OF 29 HCPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1988:88986 HCPLUS  
DOCUMENT NUMBER: 108:88986  
TITLE: Expression of **streptokinase** gene of *Streptococcus* in *Pichia pastoris*  
INVENTOR(S): Hagenson, Mary Jane; Stroman, David Womack  
PATENT ASSIGNEE(S): Phillips Petroleum Co., USA  
SOURCE: Eur. Pat. Appl., 27 pp.  
CODEN: EPXXDW

DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 248227	A1	19871209	EP 1987-106614	19870507
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
ZA 8702534	A	19871125	ZA 1987-2534	19870408
AU 8771390	A1	19871112	AU 1987-71390	19870410
AU 592862	B2	19900125		
JP 62296881	A2	19871224	JP 1987-109620	19870502
NO 8701886	A	19871109	NO 1987-1886	19870506
DK 8702335	A	19871109	DK 1987-2335	19870507
FI 8702031	A	19871109	FI 1987-2031	19870507
BR 8702337	A	19880217	BR 1987-2337	19870507
DD 257646	A5	19880622	DD 1987-302541	19870507

PRIORITY APPLN. INFO.: US 1986-860960 19860508

AB The gene for **streptokinase** of *Streptococcus equisimilis* is cloned and expressed in *Pichia pastoris*. Plasmid pHtskc25 was constructed containing the coding sequence (minus the signal sequence) for *S. equisimilis streptokinase* under the control of the alc. oxidase gene promoter of *P. pastoris*. *P. pastoris* Transformed with the plasmid and grown in MeOH-containing medium produced 16 units **streptokinase**/O.D. cells.

L6 ANSWER 22 OF 29 HCPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1986:455636 HCPLUS

DOCUMENT NUMBER: 105:55636

TITLE: The **streptokinase** gene: cloning, sequencing and expression in new hosts

AUTHOR(S): Malke, Horst

CORPORATE SOURCE: Zentralinst. Mikrobiol., Dtsch. Akad. Wiss., Jena, Ger. Dem. Rep.

SOURCE: Zeitschrift fuer Klinische Medizin (1985) (1986), 41(7), 502-4

CODEN: ZKMEEF; ISSN: 0233-1608

DOCUMENT TYPE: Journal

LANGUAGE: German

AB The **streptokinase** (I) [9002-01-1] gene (skc) of *Streptococcus equisimilis* H46A was cloned in *Escherichia coli* using vector  $\lambda$ L47. One of the recombinant clones was used to subclone skc in *E. coli* plasmid vectors. Plasmids pMF2 (10.4 kilobases, composed of pACYC184 plus a 6.4-kilobase EcoRI fragment) and pMF5 (6.9 kilobases, with a 2.5-kilobase fragment in the PstI site of pBR322) determined I formation in *E. coli*; expression of skc was independent of its orientation, indicating that the complete gene, together with its control elements, was present. The 2.5-kilobase PstI fragment of pMF5 was isolated and sequenced in the M13 system. Of 2568 base pairs, the largest open reading frame consisted of 1320 base pairs coding for prestreptokinase, corresponding to I plus its 26-amino acid leader sequence. Expression of skc was attained in *S. sanguis* after transformation with the shuttle vector pSM752. In fermentation expts., I production rates of 1500 U/mL were attained, which was below the levels obtained with *S. equisimilis*. Use of pSM752 for similar transformation of *Bacillus subtilis* is briefly discussed.

L6 ANSWER 23 OF 29 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

DUPLICATE 11

ACCESSION NUMBER: 1987:277792 BIOSIS

DOCUMENT NUMBER: PREV198784018831; BA84:18831

TITLE: MOLECULAR CLONING OF STREPTOKINASE GENE

FROM STREPTOCOCCUS-EQUISIMILIS AND ITS EXPRESSION

AUTHOR(S) : IN ESCHERICHIA-COLI.  
ROH D C [Reprint author]; KIM J H; PARK S K; LEE J W; BYRUN  
S M

CORPORATE SOURCE : DEP BIOLOGICAL SCIENCE AND ENGINEERING, KOREA ADVANCED INST  
SCIENCE AND TECHNOLOGY KAIST , PO BOX 150 CHONGRYANG, SEOUL  
131, KOREA

SOURCE: Korean Biochemical Journal, (1986) Vol. 19, No. 4, pp.  
391-398.

CODEN: KBCJAK. ISSN: 0368-4881.

DOCUMENT TYPE: Article

FILE SEGMENT: BA

LANGUAGE: ENGLISH

ENTRY DATE: Entered STN: 19 Jun 1987  
Last Updated on STN: 19 Jun 1987

AB The streptococcal genomic DNA digested with Pst I was cloned in E. coli HB101. The overlay technique of casein/plasminogen was used to screen the clones for recombinants carrying the **streptokinase** gene. The insert size of the plasmid carrying the **streptokinase** gene was a 2.5, 4.3, and 5.8 Kb, respectively. The restriction maps of all three hybrid plasmids were constructed by digestion with Pst I, Pvu II, Sal I, Hind III, Ava I, BamH I, and Cla I. For the identification of cloned gene, **streptokinase** was highly purified from *S. equisimilis* by the methods of gel chromatography and isoelectric focusing and rabbits were immunized with this purified **streptokinase**. Several lines of evidence, including proof obtained by the immunodiffusion technique, established that the enzyme from E. coli was identical to that from *S. equisimilis*. In the E. coli cell culture, we found the activity of **streptokinase** in all three principal locations of the cell. More than 50% were existed in the intracellular space.

L6 ANSWER 24 OF 29 HCPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1986:220084 HCPLUS  
DOCUMENT NUMBER: 104:220084  
TITLE: Expression of the **streptokinase** gene from *Streptococcus equisimilis* in *Bacillus subtilis*  
AUTHOR(S) : Klessen, Christian; Malke, Horst  
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Acad. Sci. GDR,  
Jena, 6900, Ger. Dem. Rep.  
SOURCE: Journal of Basic Microbiology (1986), 26, 75-81  
CODEN: JBMIEQ; ISSN: 0233-111X  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The previously cloned and sequenced **streptokinase** [9002-01-1] gene (skc) from *S. equisimilis* H46A was inserted into plasmid vectors capable of replication in *B. subtilis*. The skc gene was expressed by use of its own transcription and translation signals which appeared to meet the stringent requirements of *B. subtilis* for efficient foreign gene expression. The secreted **streptokinase** activity began to decline toward the end of the exponential growth phase suggesting that *B. subtilis* exoproteases hydrolyzed and inactivated the foreign protein.

L6 ANSWER 25 OF 29 HCPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1985:482518 HCPLUS  
DOCUMENT NUMBER: 103:82518  
TITLE: Nucleotide sequence of the **streptokinase** gene from *Streptococcus aquisimilis* H46A  
AUTHOR(S) : Malke, Horst; Roe, Bruce; Ferretti, Joseph J.  
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK,  
73190, USA  
SOURCE: Gene (1985), 34(2-3), 357-62  
CODEN: GENED6; ISSN: 0378-1119

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The entire nucleotide sequence of a cloned 2568-base-pair (bp) PstI fragment from the genome of *S. equisimilis* H46A encoding the **streptokinase** [9002-01-1] gene (skc) was determined. The longest open reading frame comprises 1320 bp which code for **streptokinase**. The protein is synthesized with a 26-amino acid residue N-terminal extension having properties characteristic of a signal peptide. Comparison of the deduced amino acid sequence with the available amino acid sequence of a com. **streptokinase** reveals minor structure differences. The nucleotide sequencing of skc does not support the hypothesis that the gene has evolved by duplication and fusion, as suggested by internal 2-fold amino acid homologies of its product. Furthermore, the skc gene sequence shows no extended regions homologous to the staphylokinase gene. Upstream from the skc gene, the putative skc promoter and the ribosome-binding site sequence were identified; downstream from the coding region, inverted repeat sequences thought to function as transcription terminators were detected.

L6 ANSWER 26 OF 29 HCPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1986:473464 HCPLUS  
DOCUMENT NUMBER: 105:73464  
TITLE: Hybridization of a cloned group C streptococcal **streptokinase** gene with DNA from other streptococcal species  
AUTHOR(S): Huang, T. T.; Malke, H.; Ferretti, J. J.  
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, USA  
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 234-6. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK.  
CODEN: 55BSAN  
DOCUMENT TYPE: Conference  
LANGUAGE: English

AB The previously cloned **streptokinase** [9002-01-1] gene (skc) of *Streptococcus equisimilis* and 2 subfragments were used as DNA hybridization probes to determine sequence homologies with other streptococcal species. The human pathogenic streptococci of strains A, C, and G were the only strains that had a pos. correlation between the ability to produce **streptokinase** and to hybridize with the gene skc DNA probe. In conjunction with other streptococcal DNA probes, such as streptolysin O, hyaluronidase, DNase, and erythrogenic toxins, the skc probe may be of diagnostic significance in the rapid identification of human pathogenic streptococci.

L6 ANSWER 27 OF 29 HCPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1986:473709 HCPLUS  
DOCUMENT NUMBER: 105:73709  
TITLE: Cloning of streptococcal genes with Streptococcus-Escherichia coli shuttle vector pSA3  
AUTHOR(S): Dao, M. L.; Ferretti, J. J.  
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, USA  
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 233-4. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK.  
CODEN: 55BSAN  
DOCUMENT TYPE: Conference  
LANGUAGE: English

AB A shuttle vector, the chimeric plasmid pSA3, which can replicate in both

E. coli and S. sanguis, was constructed. Chromosomal DNA from S. mutans was ligated into this plasmid and cloned in E. coli. Of 472 clones tested, 43 clones expressed S. mutans surface antigens. A cloned S. equisimilis streptokinase [9002-01-1] gene was inserted into plasmid pSA3 and then used to transform E. coli, S. sanguis, and S. mutans, all of which expressed the cloned streptokinase gene.

L6 ANSWER 28 OF 29 HCAPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1986:473707 HCAPLUS  
DOCUMENT NUMBER: 105:73707  
TITLE: Cloned streptokinase gene from Streptococcus equisimilis H46A  
AUTHOR(S): Malke, H.; Ferretti, J. J.  
CORPORATE SOURCE: Ger. Acad. Sci., Jena, Ger. Dem. Rep.  
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 221-2. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK.  
CODEN: 55BSAN  
DOCUMENT TYPE: Conference  
LANGUAGE: English  
AB The streptokinase [9002-01-1] gene skc of S. equisimilis was cloned in Escherichia coli with plasmid pBR322. Expression of gene skc was observed with both orientations of the gene, which indicated that its own promoter was present and was functional in E. coli. Streptokinase was excreted by the E. coli host. The gene contained a 1320-base-pair open reading frame which encodes 440 amino acids, including a signal peptide of 26 amino acids.

L6 ANSWER 29 OF 29 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
DUPLICATE 12  
ACCESSION NUMBER: 1985:232658 BIOSIS  
DOCUMENT NUMBER: PREV198579012654; BA79:12654  
TITLE: EXPRESSION OF A STREPTOKINASE GENE FROM STREPTOCOCUS-EQUISIMILIS IN STREPTOCOCUS-SANGUIS.  
AUTHOR(S): MALKE H [Reprint author]; GERLACH D; KOEHLER W; FERRETTI J  
CORPORATE SOURCE: ACAD SCI GDR, CENTRAL INST MICROBIOLOGY EXPERIMENTAL THERAPY, DDR-69 JENA, GDR  
SOURCE: Molecular and General Genetics, (1984) Vol. 196, No. 2, pp. 360-363.  
CODEN: MGGEAE. ISSN: 0026-8925.  
DOCUMENT TYPE: Article  
FILE SEGMENT: BA  
LANGUAGE: ENGLISH  
AB Using recombinant DNA techniques, one introduced a previously cloned streptokinase gene from S. equisimilis into the Challis strain of S. sanguis (group H). The gene was expressed in the new host under the control of its own promoter and the gene product had biological properties identical to authentic streptokinase. The MW of cloned streptokinase (42 K [kilodalton]) as expressed by S. sanguis was substantially lower than that of authentic streptokinase (47 K). Since the cloned streptokinase gene encoded a 47 K mature protein, the lowered MW of S. sanguis streptokinase may reflect posttranslational proteolytic cleavage, which leaves the biological activity of the gene product and its serological reactivity unimpaired.

=> s inclusion (a) bod?  
L7 44795 INCLUSION (A) BOD?

=> d his

(FILE 'HOME' ENTERED AT 09:47:34 ON 11 MAY 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004

L1 40597 S STREPTOKINASE?  
L2 362 S "S. EQUISIMILIS"  
L3 103 S L1 AND L2  
L4 6513995 S CLON? OR EXPRESS? OR RECOMBINANT  
L5 74 S L3 AND L4  
L6 29 DUP REM L5 (45 DUPLICATES REMOVED)  
L7 44795 S INCLUSION (A) BOD?

=> s 16 and 17

L8 1 L6 AND L7

=> d all

L8 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
AN 2000:96119 BIOSIS  
DN PREV200000096119  
TI Two **streptokinase** genes are **expressed** with different solubility in Escherichia coli W3110.  
AU Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens, Isis  
CS Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba  
SO Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.  
CODEN: BILED3. ISSN: 0141-5492.  
DT Article  
LA English  
ED Entered STN: 15 Mar 2000  
Last Updated on STN: 3 Jan 2002  
AB The **streptokinase** (SK) gene from **S. equisimilis** H46A (ATCC 12449) was **cloned** in **E. coli** W3110 under the control of the tryptophan promoter. The **recombinant** SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from **S. equisimilis** (ATCC 9542) which had been **cloned** in **E. coli** W3110 by using similar **expression** vector and cell growth conditions, and occurred in the form of **inclusion bodies**.  
CC Genetics of bacteria and viruses 31500  
Biochemistry methods - Nucleic acids, purines and pyrimidines 10052  
Biochemistry methods - Proteins, peptides and amino acids 10054  
Replication, transcription, translation 10300  
Biophysics - Molecular properties and macromolecules 10506  
Microbiological apparatus, methods and media 32000  
Food microbiology - General and miscellaneous 39008  
Enzymes - General and comparative studies: coenzymes 10802  
Metabolism - Proteins, peptides and amino acids 13012  
Morphology and cytology of bacteria 30500  
Physiology and biochemistry of bacteria 31000  
IT Major Concepts  
Enzymology (Biochemistry and Molecular Biophysics); Molecular Genetics (Biochemistry and Molecular Biophysics)  
IT Chemicals & Biochemicals  
amino acids; enzymes; proteins; tryptophan  
IT Miscellaneous Descriptors  
biotechnology; cell growth conditions; expression vectors; gene expression; promoters; tryptophan promoter

ORGN Classifier  
Enterobacteriaceae 06702  
Super Taxa  
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria;  
Microorganisms  
Organism Name  
Escherichia coli: W 3110  
Taxa Notes  
Bacteria, Eubacteria, Microorganisms  
ORGN Classifier  
Gram-Positive Cocci 07700  
Super Taxa  
Eubacteria; Bacteria; Microorganisms  
Organism Name  
Streptococcus equisimilis  
Taxa Notes  
Bacteria, Eubacteria, Microorganisms  
RN 54-12-6Q (tryptophan)  
73-22-3Q (tryptophan)

=> s "lambdapr"  
L9 42 "LAMBDAPR"  
  
=> d his  
(FILE 'HOME' ENTERED AT 09:47:34 ON 11 MAY 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,  
LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004  
L1 40597 S STREPTOKINASE?  
L2 362 S "S. EQUISIMILIS"  
L3 103 S L1 AND L2  
L4 6513995 S CLON? OR EXPRESS? OR RECOMBINANT  
L5 74 S L3 AND L4  
L6 29 DUP REM L5 (45 DUPLICATES REMOVED)  
L7 44795 S INCLUSION (A) BOD?  
L8 1 S L6 AND L7  
L9 42 S "LAMBDAPR"

=> s l2 and l9  
L10 0 L2 AND L9

=> s l1 and l9  
L11 0 L1 AND L9

=> s l9(w) lambdapl  
L12 0 L9(W) LAMBDAPL

=> s "lambdapr-lambdapl"  
L13 0 "LAMBDAPR-LAMBDAPL"

=> dup rem l9  
PROCESSING COMPLETED FOR L9  
L14 35 DUP REM L9 (7 DUPLICATES REMOVED)

=> d 1-10 ibib ab

L14 ANSWER 1 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 2004:147086 BIOSIS  
DOCUMENT NUMBER: PREV200400151628  
TITLE: Gene expression patterns controlled by a temperature  
sensitive promoter.  
AUTHOR(S): Chapman, Emily [Reprint Author]; Wu, Xiao-lun [Reprint]

CORPORATE SOURCE: Author]  
Physics and Astronomy, University of Pittsburgh,  
Pittsburgh, PA, USA  
SOURCE: Biophysical Journal, (January 2004) Vol. 86, No. 1, pp.  
420a. print.  
Meeting Info.: 48th Annual Meeting of the Biophysical  
Society. Baltimore, MD, USA. February 14-18, 2004.  
Biophysical Society.  
ISSN: 0006-3495 (ISSN print).  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 17 Mar 2004  
Last Updated on STN: 17 Mar 2004

AB Gene expression patterns are not very well understood even in simple biological systems. This was investigated using a promoter that is repressed by the CI857 protein. We cloned GFPmut3 into a vector where the expression of the protein is controlled by the PL promoter, and the transcription of the repressor is regulated by the **lambdaPR** promoter. When the temperature of a sample reaches approximately 37degreeC the repressor protein is no longer able to bind and the PL promoter can begin expression of the GFP. We used the fluorescence properties of GFP to estimate the average amount of protein expressed in a large population of *E. coli*, as well as the expression level of individual cells. It was found that for minimally induced cells, there is a great deal of heterogeneities between different cells similar to those observed in the well-studied pBAD system. A simple model is constructed to explain the gene expression patterns seen in the large population and in individual cells. This temperature sensitive promoter can be a tool in the study of the dynamics of a synthetic genetic network.

L14 ANSWER 2 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 2004:147069 BIOSIS  
DOCUMENT NUMBER: PREV200400151620  
TITLE: Urea as a probe of conformational changes in transcription initiation for *E. coli* RNA Polymerase.  
AUTHOR(S): Kontur, Wayne S. [Reprint Author]; Davis, Caroline;  
Saecker, Ruth [Reprint Author]; Record, M. Thomas Jr.  
CORPORATE SOURCE: Chemistry, University of Wisconsin-Madison, Madison, WI,  
USA  
SOURCE: Biophysical Journal, (January 2004) Vol. 86, No. 1, pp.  
418a. print.  
Meeting Info.: 48th Annual Meeting of the Biophysical  
Society. Baltimore, MD, USA. February 14-18, 2004.  
Biophysical Society.  
ISSN: 0006-3495 (ISSN print).  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 17 Mar 2004  
Last Updated on STN: 17 Mar 2004

AB Binding of *E. coli* RNA Polymerase Holoenzyme (R) to **lambdaPR** promoter DNA (P) to form an open complex (R<sub>P</sub>) follows a three-step mechanism involving two intermediates, I<sub>1</sub> and I<sub>2</sub>. Footprinting studies have been used to characterize the state of the DNA in I<sub>1</sub> and R<sub>P</sub>, but probes are needed to assay conformational changes that take place in the protein throughout the process. Urea effects (like dCpo effects) should be a useful diagnostic of local folding or unfolding in the steps of a mechanism. The effect of urea concentration on the equilibrium constant for protein unfolding is correlated with the amount of protein surface area exposed to the solvent (dASA) upon unfolding (Myers et al.:, 95; Courtenay et al., 00): m-value=RTdlnKobs/d(urea)=0.14dASA. This same analysis can be applied to a kinetic process, where the rate constant of a step is related to dASA for formation of the transition state of the step.

From studying the urea dependence of the kinetics of the association and dissociation of polymerase and promoter DNA, we have determined that all three steps exhibit characteristic urea dependences, which provide evidence for coupled folding or unfolding of the enzyme. From the urea data, which is consistent with and expands upon previous dCpo data, we obtain evidence for large-scale folding in forming I1, and especially in forming RPO from the (I1-I2) transition state. Interestingly, the m-value for the conversion of I1 to this crucial, rate-determining transition state is negative, arguing for unfolding in this step. Structural proposals for these coupled folding/unfolding events will be presented. This type of analysis can be applied to other systems to help develop a molecular picture for the mechanism of a reaction.

L14 ANSWER 3 OF 35 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN  
ACCESSION NUMBER: 2004:327257 SCISEARCH  
THE GENUINE ARTICLE: 806TB  
TITLE: Differential expression of human basic fibroblast growth factor in Escherichia coli: potential role of promoter  
AUTHOR: Mirzahoseini H (Reprint); Mehraein F; Omidinia E; Razavi M R  
CORPORATE SOURCE: Pasteur Inst Iran, Dept Biochem, Pasteur St 69, Tehran 13164, Iran (Reprint); Pasteur Inst Iran, Dept Biochem, Tehran 13164, Iran  
COUNTRY OF AUTHOR: Iran  
SOURCE: WORLD JOURNAL OF MICROBIOLOGY & BIOTECHNOLOGY, (MAR 2004) Vol. 20, No. 2, pp. 161-165.  
Publisher: KLUWER ACADEMIC PUBL, VAN GODEWIJCKSTRAAT 30, 3311 GZ DORDRECHT, NETHERLANDS.  
ISSN: 0959-3993.

DOCUMENT TYPE: Article; Journal  
LANGUAGE: English

REFERENCE COUNT: 15

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Four different expression systems were developed for expression of the cDNA encoding human basic fibroblast growth factor ( hbFGF), using Escherichia coli as host organism. The hbfgf structural gene was cloned into four expression vectors, pET-3a, pTrc99A, pPR37 and pKK223-3 differing only in their promoters, which were T7, trc, lambdaPR and tac respectively. Expression of the gene was induced by adding 0.5 mM (final concentration) of isopropyl-beta-D- thio-galactopyranoside ( IPTG) for the vectors carrying T7, trc and tac promoters or by a temperature shift from 30 to 42 degreesC for the vector carrying kPR. The highest level of expression was observed in pET-1005 ( a pET-3a derivative)/BL21 (DE3) system with 18.5 mg/l rhbFGF and the second high level expression was in pR37-1007 ( pPR37 derivative) BL21 (DE3) system with 5 mg of rhbFGF/l. Since in the latter system a temperature shift was used for induction, 29% of the hbFGF was recovered as inclusion bodies in the insoluble cell fraction. The level of expression for the two other systems (pTrc-1006 and pKK-1008) was very low.

L14 ANSWER 4 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
ACCESSION NUMBER: 2004-05827 BIOTECHDS

TITLE: Producing recombinant virus protein R (Vpr), useful in promoting immunodeficiency virus reproduction, involves transforming a host cell with an expression vector for the protein;

recombinant protein production via plasmid expression in host cell for use in immunodeficiency virus reproduction

PATENT ASSIGNEE: ORIENTAL YEAST CO LTD; NAKAMURA T

PATENT INFO: JP 2003259881 16 Sep 2003

APPLICATION INFO: JP 2002-66938 12 Mar 2002

PRIORITY INFO: JP 2002-66938 12 Mar 2002; JP 2002-66938 12 Mar 2002

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

OTHER SOURCE: WPI: 2004-026590 [03]

AB DERWENT ABSTRACT:

NOVELTY - Producing (M1) recombinant virus protein R (Vpr) protein (I) having a fully defined sequence (S1) of 96 amino acids as given in the specification, or a sequence having one or more substitutions, additions or alterations in (S1) and having biological activity, by transforming a host Escherichia coli cell (II) with an expression vector (III) containing the gene encoding (I), and culturing the transformed cell, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) the expression vector (III) useful in M1; (2) an Escherichia coli cell (II) transformed by vector (III); (3) recombinant virus protein R (I) produced by M1; and (4) promoting (M2) immunodeficiency virus reproduction in a cell, tissue or organ derived from an organism by administering (I).

BIOTECHNOLOGY - Preferred Method: In M1, transformed cell (II) is N4830-1. (III) is p **lambdaPR**-A2 having an accession number FERM P-18748. (I) does not contain another heterologous protein or peptide except Vpr protein. In M2, the cell is a chronic sustainable virus infected cell.

USE - M1 is useful for producing recombinant Vpr protein. (I) is useful in method M2 for promoting the reproduction of immunodeficiency virus in cell, tissue or organ derived from an organism (all claimed). (I) is useful for functional analysis of the Vpr protein.

ADVANTAGE - (I) is produced efficiently by (M1) at a low cost. Functional analysis showed that Vpr enhances reproduction of HIV-1 in chronic sustainable infected U1 cell. The effect of Vpr is neutralized by anti tumor necrosis factor (TNF) alpha antibodies.

EXAMPLE - Recombinant Vpr protein was produced as follows. Recombinant full-length Vpr protein was produced by the Escherichia coli expression system. The full-length sequence of Vpr was amplified by PCR. The natural Vpr gene was mutated. The acquired PCR products were digested by the restriction enzyme NcoI and PstI and coupled with the expression vector p **lambdaPR** and p **lambdaPR**-A2 was built. The expression of a Vpr gene, which contains temperature sensitivity to lambda c1857 was used as a host cell. The transformed organisms were collected. The purified transformed organisms were cultured. The recombinant Vpr protein was purified from the supernatant liquid which contains transformed microbial cells. The produced recombinant protein was analyzed by Western blotting. The results confirmed the production of 15 KDa recombinant protein. The amount of recombinant protein produced was high. The function of Vpr in the enhancement of reproduction of HIV1 in chronic sustainable virus infected cell was studied by administering the recombinant Vpr protein. The results showed that Vpr had a specific role in the enhancement of reproduction of HIV1. The effect was similar to the function of tumor necrosis factor (TNF) alpha. The effect of the Vpr was neutralized by the anti TNF alpha antibodies. (14 pages)

L14 ANSWER 5 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:500115 BIOSIS

DOCUMENT NUMBER: PREV200300502236

TITLE: Efficient biosynthetic incorporation of tryptophan and indole analogs in an integral membrane protein.

AUTHOR(S): Broos, Jaap [Reprint Author]; Gabellieri, Edi; Biemans-Oldehinkel, Esther; Strambini, Giovanni B.

CORPORATE SOURCE: Department of Biochemistry, Groningen Biomolecular Science and Biotechnology Institute (GBB), University of Groningen, Nijenborgh 4, 9747 AG, Groningen, Netherlands  
J.Broos@chem.rug.nl

SOURCE: Protein Science, (September 2003) Vol. 12, No. 9, pp. 1991-2000. print.

ISSN: 0961-8368.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 29 Oct 2003  
Last Updated on STN: 29 Oct 2003

AB Biosynthetic incorporation of tryptophan (Trp) analogs such as 7-azatryptophan, 5-hydroxytryptophan, and fluorotryptophan into a protein can facilitate its structural analysis by spectroscopic techniques such as fluorescence, phosphorescence, nuclear magnetic resonance, and Fourier transform infrared. Until now, the approach has dealt primarily with soluble proteins. In this article, we demonstrate that four different Trp analogs can be very efficiently incorporated into a membrane protein as demonstrated for the mannitol transporter of *Escherichia coli* (EIImtl). EIImtl overexpression was under control of the **lambdaPR** promoter, and the *E. coli* Trp auxotroph M5219 was used as host. This strain constitutively expresses the heat labile repressor protein of the **lambdaPR** promoter. Together with the presence of the repressor gene on the EIImtl plasmid, this resulted in a tightly controlled promoter system, a prerequisite for high Trp analog incorporation. A new method for determining the analog incorporation efficiency is presented that is suitable for membrane proteins. The procedure involves fitting of the phosphorescence spectrum as a linear combination of the Trp and Trp analog contributions, taking into account the influence of the protein environment on the Trp analog spectrum. The data show that the analog content of EIImtl samples is very high (>95%). In addition, we report here that biosynthetic incorporation of Trp analogs can also be effected with less expensive indole analogs, which *in vivo* are converted to L-Trp analogs.

L14 ANSWER 6 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 2003:348497 BIOSIS  
DOCUMENT NUMBER: PREV200300348497  
TITLE: Interplay between DnaA and SeqA proteins during regulation of bacteriophage **lambdaPR** promoter activity.  
AUTHOR(S): Slominska, Monika; Konopa, Grazyna; Baranska, Sylwia; Wegrzyn, Grzegorz; Wegrzyn, Alicja [Reprint Author]  
CORPORATE SOURCE: Laboratory of Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, University of Gdansk, Kladki 24, 80-822, Gdansk, Poland  
wegrzyn@biotech.univ.gda.pl  
SOURCE: Journal of Molecular Biology, (23 May 2003) Vol. 329, No. 1, pp. 59-68. print.  
ISSN: 0022-2836 (ISSN print).  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 30 Jul 2003  
Last Updated on STN: 30 Jul 2003

AB DnaA and SeqA proteins are main regulators (positive and negative, respectively) of the chromosome replication in *Escherichia coli*. Nevertheless, both these replication regulators were found recently to be also transcription factors. Interestingly, both DnaA and SeqA control activity of the bacteriophage **lambdaPR** promoter by binding downstream of the transcription start site, which is unusual among prokaryotic systems. Here we asked what are functional relationships between these two transcription regulators at one promoter region. Both *in vivo* and *in vitro* studies revealed that DnaA and SeqA can activate the PR promoter independently and separately rather than in co-operation, however, increased concentrations of one of these proteins negatively influenced the transcription stimulation mediated by the second regulator. This may suggest a competition between DnaA and SeqA for binding to the PR regulatory region. The physiological significance of this DnaA and SeqA-mediated regulation of PR is demonstrated by studies on lambda plasmid DNA replication *in vivo*.

L14 ANSWER 7 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
ACCESSION NUMBER: 2003-07812 BIOTECHDS  
TITLE: New vector comprising 2 or more genes encoding

sugar-nucleotide regenerating enzymes and one or more genes encoding glycosyltransferases, useful for producing glycoconjugates, including oligosaccharides in large-scale; involving vector-mediated gene transfer and expression in host cell for use in oligonucleotide synthesis

AUTHOR: WANG P G; CHEN X; LIU Z; ZHANG W

PATENT ASSIGNEE: WANG P G; CHEN X; LIU Z; ZHANG W

PATENT INFO: US 2002132320 19 Sep 2002

APPLICATION INFO: US 2001-758525 10 Jan 2001

PRIORITY INFO: US 2001-758525 10 Jan 2001; US 2001-758525 10 Jan 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-165735 [16]

AB DERWENT ABSTRACT:

NOVELTY - Vector comprising: (a) two or more genes encoding sugar-nucleotide regenerating enzymes selected from GalK, GalKT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding glycosyltransferase(s), is new. The genes are operably linked to a promoter.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell comprising heterologous genes encoding one or more sugar-nucleotide regenerating enzyme and one or more glycosyltransferase; (2) producing a glycoconjugate by contacting a cell comprising heterologous genes encoding: (a) 2 or more genes encoding sugar-nucleotide regenerating enzymes selected from GalK, GalKT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, Pgm, NagE, Agm1, glum, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and (b) one or more genes encoding glycosyltransferase(s), with a bioenergetic; (3) a kit comprising the plasmid; and (4) a non-human cell comprising the plasmid.

WIDER DISCLOSURE - Disclosed are the following: (1) producing sugar nucleotides; (2) organisms engineered to express sugar nucleotide regeneration enzymes and/or glycosyltransferase enzymes; and (3) systems for producing glycoconjugates and sugar nucleotides.

BIOTECHNOLOGY - Preferred Vector: The vector comprises genes encoding 3 or more enzymes for regenerating a sugar-nucleotide, and genes encoding 2 or more glycosyltransferases. The vector comprises genes encoding GalK, GalT and GalU, and a gene encoding Ndk, Ppk, PykF, PoxB, Ndk, Ppa, SusA, GalE, GluT, Ugd or UGT2B7. The glycosyltransferases is selected from a galactosyltransferase, a glucosyltransferase, an N-acetylglucosaminyl transferase, a sialyltransferase, a mannosyltransferase, and a fucosyltransferase. The galactosyltransferase is LgtB or LgtC. The glucosyltransferase is LgtF, Alg5, or DUGT. The N-acetylglucosaminyltransferase is UDP-GalNAc:2'-fucosylgalactoside-alpha-3-N-acetylgalactosaminyl transferase. The glucuronyltransferase is UGT2B7. The sialyltransferase is SiaT 0160. The mannosyltransferase is Alfl or alg2. The fucosyltransferase is alpha1,3-FucT, alpha1,2-FucT or alpha1,3/4-FucT. The promoter is an inducible promoter, preferably lambdaPR promoter, and further comprises a lambda C 1 repressor gene. At least one gene is operably linked to a ribosomal binding site, to an IRES, or to a tag sequence. Each gene encoding a sugar-nucleotide regenerating enzyme or a glycosyltransferase is operably linked to a ribosomal binding site sequence or to a tag sequence encoding polyhistidine. The vector encodes an epimerase or a fusion protein comprising an epimerase and a glycosyltransferase, where the epimerase is UDP-Gal-4-epimerase and the glycosyltransferase is an alpha-1,3-galactosyltransferase. The vector is selected from plasmids, phages, phagemids, viruses, and artificial chromosomes, preferably a plasmid. Preferred Cell: The cell can be a prokaryotic or a eukaryotic cell. The prokaryotic cell is a bacterium preferably E. coli LacZ-. The eukaryotic cell is a yeast cell. One of the heterologous genes is integrated into the genome of the cell. The heterologous genes are

encoded within one plasmid.

USE - The vector is useful for large-scale synthesis of glycoconjugates, including oligosaccharides. (51 pages)

L14 ANSWER 8 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
ACCESSION NUMBER: 2003-02526 BIOTECHDS

TITLE: Novel isolated protein useful for identify a compound capable of inhibiting astacin metalloproteinase activity of a parasite;  
recombinant metallo protease production and drug screening useful for parasite infection therapy and vaccine preparation

AUTHOR: TRIPP C A; FRANK G R; GRIEVE R B

PATENT ASSIGNEE: HESKA CORP

PATENT INFO: US 2002086974 4 Jul 2002

APPLICATION INFO: US 2001-864541 23 May 2001

PRIORITY INFO: US 2001-864541 23 May 2001; US 1994-249552 26 May 1994

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-665480 [71]

AB DERWENT ABSTRACT:

NOVELTY - An isolated protein (I) comprising a parasite astacin metalloendopeptidase protein, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated antibody (II) capable of selectively binding to (I); (2) a therapeutic composition (III) for protecting an animal from disease caused by a parasite, the parasite being susceptible to an inhibitor of an astacin metalloendopeptidase, the therapeutic composition comprising at least one protective compound selected from an isolated parasite astacin metalloendopeptidase protein, an anti-parasite astacin metalloendopeptidase antibody, and an inhibitor of astacin metalloendopeptidase activity identified by its ability to inhibit parasite astacin metalloendopeptidase activity of the protein; and (3) a test kit to identify a compound capable of inhibiting astacin metalloendopeptidase activity of a parasite, the test kit comprises an isolated parasite astacin metalloendopeptidase activity and a method for determining the extent of inhibition of the activity in the presence of a putative inhibitory compound.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing in a recombinant cell, transformed with a nucleic acid molecule encoding the protein to produce the protein (claimed). Preferred Protein: If (I) is administered to an animal in an effective manner, it elicits an immune response against a parasite astacin metalloendopeptidase, where the parasite is parasitic helminths, protozoan parasites, ectoparasites, nematodes, cestodes or trematodes. The parasite comprises a tissue-migrating helminth or a nematode selected from filariid (such as *Dirofilaria*, *Acanthocheilonema*, *Brugia*, *Dipetalonema*, *Loa*, *Onchocerca*, *Parafilaria*, *Setaria*, *Stephanofilaria* and *Wuchereria* filariid nematodes), ascarid, strongyle and trichostrongyle nematodes. The parasite comprises *Dirofilaria immitis*. (I) is encoded by a parasite nucleic acid molecule which hybridizes under stringent conditions with *D. immitis* astacin metalloendopeptidase gene. (I) comprises an amino acid sequence having at least 40% homology with an amino acid sequence selected from L3 nDiMPA32076 and adult nDiMPA32028. (I) comprises at least a portion of an amino acid sequence, where the portion is encoded by a nucleic acid molecule which hybridizes under stringent conditions with a nucleic acid molecules selected from nDiMPA11299, nDiMPA22126, L3 nDiMPA32292, L3 nDiMPA32076, adult nDiMPA32032, and adult nDiMPA32028. (I) comprises an extended zinc-binding domain motif. Preferred Composition: (III) further comprises at least one component selected from an excipient, adjuvant and a carrier.

ACTIVITY - Antiparasitic; Antihelminthic.

MECHANISM OF ACTION - Inhibitor of astacin metalloproteinase activity; Vaccine. No supporting data is given.

USE - (I) is useful to identify an inhibitor of astacin metalloendopeptidase activity. (III) is useful to protect an animal from disease such as heartworm infection, caused by a parasite, by administering (III) to an animal. (I) is useful to identify a compound capable of inhibiting astacin metalloproteinase activity of a parasite, by contacting (I) with a putative inhibitory compound under conditions in which, in the absence of the compound, the astacin metalloendopeptidase protein has astacin metalloendopeptidase activity, and determining if the putative inhibitory compound inhibits the activity. (All claimed). (I) is useful to treat, ameliorate and/or prevent disease, caused by parasite. (II) is useful as vaccines to passively immunize an animal to protect the animal from parasite susceptible to treatment by such antibodies. (II) is useful as reagents in assays to detect infection by parasites and as tools to recover desired proteins from a mixture of proteins and other contaminants. (II) is also useful as immunotherapeutic agents and to target cytotoxic agents to parasites to directly kill the parasites.

ADMINISTRATION - (III) is administered by subcutaneous, intradermal, intravenous, nasal, oral, transdermal or intramuscular route. (III) is administered at a dose rate of 1 microg-10 mg/kg. Vaccine is administered at a dose of 10 microg-1 mg/kg.

EXAMPLE - Recombinant molecule plambdaPRHis-nDiMPA2804, containing nucleotides from about positions 119 through 922 of nDiMPA22126 operatively linked to a lambdaPR transcription control sequences and to a fusion sequence encoding a poly-histidine segment comprising 6 histidines was produced. Nucleic acid molecule nDiMPA2804 was ligated. The vector which was 3455 base pairs contained 1990 base pair PvII to AatII fragment from pUC19 containing the ampicillin resistance gene and Escherichia coli origin of replication, 1100 base pair BgIII to BgIII DNA fragment from vector pRK248cIts with a PvuII linker added to one end, containing the lambdaPR promoter, the cI857 lambda repressor gene and 22 amino acid of the cro gene regulating lytic growth, a 55 base pair BglII to XbaI segment from pGEMEX-1 which contained the T7 promoter, a 170 base pair XbaI to ECORI segment from pRSET-B which contained the T7-S10 translational enhancer, the His6 fusion, the 11 amino acid S10 leader fusion, an enterokinase cleavage site and the multiple cloning site, and a 140 base pair fragment containing synthetic translational and transcription termination signals including the T1 translation terminators in all 3 reading frames, RNA stabilization sequence from Bacillus thurengiensis crystal protein and the T2 rho-independent transcription terminator from the trpA operon. The resulting recombinant molecule denoted plambdaPRHis-nDiMPA2804 was transformed into E. coli to form recombinant cell E. coli:plambdaPRHis-nDiMPA2804. (27 pages)

L14 ANSWER 9 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
ACCESSION NUMBER: 2003-06044 BIOTECHDS

TITLE: Novel Herpesviridae thymidine kinase mutant useful for inhibiting pathogens e.g. viruses, bacteria, tumor in animals, has one or more mutations encoding amino acid substitutions upstream from the DRH nucleoside binding site; herpes simplex virus thymidine-kinase production and its encoding gene useful for gene therapy

AUTHOR: LOEB L A; BLACK M E

PATENT ASSIGNEE: UNIV WASHINGTON

PATENT INFO: US 6451571 17 Sep 2002

APPLICATION INFO: US 1999-270956 17 Mar 1999

PRIORITY INFO: US 1999-270956 17 Mar 1999; US 1994-237592 2 May 1994

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-045581 [04]

AB DERWENT ABSTRACT:

NOVELTY - Isolated Herpesviridae thymidine kinase (TK) comprising a 12 amino acid (aa) nucleoside binding region having a site 3 made up of a DRH motif and a site 4 and mutation(s), at least one of the mutations

being an aa substitution 2 or 3 aa upstream or 5 or more aa downstream from DRH motif that increases a biological activity, preferably ability of TK to phosphorylate a nucleoside analog, as compared to unmutated TK, is new.

DETAILED DESCRIPTION - The mutant TK comprises a 12 aa nucleoside binding region having a site 3 made up of a DRH motif and a site 4 and mutation(s), at least one of the mutations being an aa substitution 2 or 3 amino acids upstream from DRH motif or a methionine or tyrosine substitution 5 amino acids downstream from the DRH motif, that increases a biological activity, preferably the ability of TK to phosphorylate a nucleoside analog, as compared to unmutated TK, such that  $Z$  is lesser than:  $((TKmNAP)/(TKmTp))/((TKwtNAP)/(TKwtTp))$   $TKmNAP$  = the rate of phosphorylation of a nucleoside analog by a TK mutant;  $TKm\ Tp$  = the rate of phosphorylation of thymidine by a TK mutant;  $TKwt\ NAP$  = the rate of phosphorylation of a nucleoside analog by an unmutated TK enzyme;  $TKwt\ Tp$  = the rate of phosphorylation of a TK enzyme by an unmutated TK enzyme; and  $z$  = at least 1.

BIOTECHNOLOGY - Preferred Mutant: TK is Varicella Zoster virus, Herpes Simplex virus type 1 or type 2 TK. and is truncated or contains an in-frame deletion.  $z$  is 1.5, 2, 2.5, 3, 3.5, 4, 4.5 or 5. The nucleoside analog is gancyclovir, acyclovir, buycyclovir, famcyclovir, pencyclovir, valacyclovir, trifluorothymidine, 1-(2-deoxy, 2-fluoro, beta-D-arabino furanosyl)-5-iodouracil, ara-A, araT, 1-beta-D-arabinofuranosyl thymine, 5-ethyl-2'-deoxyuridine, 5-ido-5'-amino-2,5'-dideoxyuridine, idoxuridine, AZT, AIU, dideoxycytidine or AraC. The mutant TK comprises one or more mutations, at least one of the mutations being a cysteine substitution 6 amino acids downstream from the DRH motif, or at least 5 mutations that increase a biological activity of TK as compared to unmutated TK, including a mutation to isoleucine 3 aa upstream from DRH motif, a mutation to leucine 2 aa upstream from the DRH motif, a mutation to alanine one aa upstream from the DRH motif, a mutation to tyrosine 4 aa downstream from DRH motif and a mutation to phenylalanine 5 aa downstream from DRH motif. Alternatively the mutation is at least 4 mutations including a mutation to leucine 1 or 2 aa upstream from DRH motif, a mutation to valine 4 aa upstream from DRH motif and a mutation to methionine 5 aa downstream from DRH motif; or 4 mutations including a mutation to leucine 1 aa upstream from the DRH motif, a mutation to serine 4 aa downstream, a mutation to tyrosine 5 aa downstream and a mutation to cysteine 6 aa downstream from the DRH motif.

ACTIVITY - Virucide; Antibacterial; Antiparasitic; Cytostatic; Antipsoriatic; Immunosuppressive; Antiallergic; Vasotropic; Anti-HIV; Nootropic; Neuroprotective; Keratolytic. No biological data.

MECHANISM OF ACTION - Gene therapy.

USE - TK mutants are useful for inhibiting a pathogenic agent such as viruses, bacteria, parasites, tumor cells or autoreactive immune cells in a warm-blooded animal. TK mutant is useful for inhibiting a tumor or cancer in a warm-blooded animal, for treating a variety of disease e.g., hyperkeratosis (psoriasis), prostate hypertrophy, hyperthyroidism, endocrinopathies, autoimmune diseases, allergies, restenosis, viral diseases such as acquired immunodeficiency syndrome (AIDS) hepatitis (HCV or HBV), intracellular parasitic diseases, and to correct aberrant expression of a gene within a cell, or to replace a specific gene which is defective in proper expression, e.g. including adenosine deaminase deficiency, and Alzheimer's diseases. The mutants are utilized as a conditionally lethal marker for homologous recombination.

ADMINISTRATION - Administered by intraarticular, intracranial, intradermal, intramuscular, intraocular, intraperitoneal, intrathecal, intravenous, subcutaneous, or even directly into a tumor. Dosage details not given.

EXAMPLE - A 52-mer oligonucleotide with a wild-type thymidine kinase (tk) sequence and a 56-mer that contained degenerate nucleotides spanning from codon 165-175 of the tk gene were synthesized. Chimeric vectors pMDC (which produces an inactive TK gene product) and pMCC (which produces wild-type TK) were produced from plasmids pHETK1 and pHETK2 which

contained a HSV-1 tk structural gene, and were derivatives of pBR322. Plasmid pHETK2 contained lambdaPL and **lambdaPR** promoters, ampR, and the c1857 temperature sensitive repressor, where pHETK1 contained all the above except the lambdaPL promoter. To construct pMDC and pMCC, a dummy vector, designated pKTPD was first constructed using oligonucleotides (O1) and (O2), which were phosphorylated and then annealed. pHETK2 was digested with SstI and KpnI restriction endonucleases, and the large fragment isolated. Two picomoles of the large fragment was ligated with 6 pmol of the double-stranded oligonucleotide. The resultant double-stranded circular DNA product (designated pKTPD) was used to transform competent Escherichia coli KY895 cells. pHETK1 and pKTPD were then utilized to construct a new chimeric dummy vector, designated pMDC that produced an inactive tk gene product. Another chimeric vector, pMCC, containing the wild-type tk gene was similarly constructed by ligating the larger fragment from pHETK1 with the smaller fragment of pHETK2. pMCC produces active wild-type TK. A library containing 20% random nucleotide sequences was constructed. A 52-mer oligo containing wild-type sequences was hybridized to a 56-mer oligo which contained degenerate sequences spanning codons 165-175. The hybrid was extended with the Klenow fragment of E. coli DNA polymerase I to produce a complete double-stranded DNA product. The Klenow fragment generated double-stranded DNA was then subjected to polymerase chain reaction (PCR) amplification and the amplified DNA was digested with KpnI and SacI. The digested double-stranded oligonucleotide containing the random sequence was again purified and ligated to the KpnI/SacI digested large fragment of pMDC and used to transform competent E. coli KY895 by electroporation. An aliquot of each transformation solution was spread onto Luria Bertani (LB)-agar medium containing 50 micrograms/ml of 5'-fluorodeoxyuridine, 2 micrograms/ml of thymidine, 20 micrograms/ml of carbenicillin to determine total number of transformants. Selection for active TK clones was performed on TK selection medium that contained 50 micrograms/ml of carbenicillin, 10 micrograms/ml of 5' fluorodeoxyuridine, 2 micrograms/ml of thymidine, 20 micrograms/ml of uridine, 2% BBL peptone, 0.5% NaCl, 0.2% glucose, and 0.8% Gel-Rite. Colonies on carbenicillin medium was incubated at 37degreesC for 14-16 hours, whereas inoculated TK selection medium was incubated at 37degreesC for 24 hours. From a total of 52000 transformants that grew on carbenicillin medium, 190 were able to complement E. coli KY895 for TK function. CCCCTCGAGCGCGGTAC (O1) CGCGCTCGAGGGGAGCT (O2) (63 pages)

L14 ANSWER 10 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 2003:43915 BIOSIS  
DOCUMENT NUMBER: PREV200300043915  
TITLE: Multiple mechanisms of transcription inhibition by ppGpp at the **lambdaPR** promoter.  
AUTHOR(S): Potrykus, Katarzyna; Wegrzyn, Grzegorz; Hernandez, V. James [Reprint Author]  
CORPORATE SOURCE: Dept. of Microbiology, State University of New York, Buffalo, NY, 14214, USA vjh@buffalo.edu  
SOURCE: Journal of Biological Chemistry, (November 15 2002) Vol. 277, No. 46, pp. 43785-43791. print.  
CODEN: JBCHA3. ISSN: 0021-9258.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 15 Jan 2003  
Last Updated on STN: 15 Jan 2003  
AB General stress conditions in bacterial cells cause a global cellular response called the stringent response. The first event in this control is production of large amounts of a regulatory nucleotide, guanosine-3',5'-(bis)pyrophosphate (ppGpp). It was proposed recently that ppGpp acts by decreasing stability of open complexes at promoters that make short-lived open complexes, e.g. the rRNA promoters. However, here we report that the bacteriophage **lambdaPR** promoter, which forms

long-lived open complexes, is inhibited by ppGpp in vitro as observed in vivo. We performed a systematic investigation of the ppGpp-specific inhibition of transcription initiation at **lambdapR** and found that ppGpp does decrease stability of open complexes at **lambdapR**, but only slightly. Likewise the equilibrium binding constant and rate of open complex formation by RNA polymerase at **lambdapR** are only slightly affected by ppGpp. The major effect of ppGpp-mediated inhibition is to decrease the rate of promoter escape. We conclude that ppGpp-mediated inhibition of transcription initiation is not restricted to promoters that make short-lived open complexes. Rather we conclude that the initial catalytic step of transcript formation is affected by ppGpp, specifically formation of the first phosphodiester bond is inhibited by ppGpp at **lambdapR**.

=> d his

(FILE 'HOME' ENTERED AT 09:47:34 ON 11 MAY 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004

L1        40597 S STREPTOKINASE?  
L2        362 S "S. EQUISIMILIS"  
L3        103 S L1 AND L2  
L4        6513995 S CLON? OR EXPRESS? OR RECOMBINANT  
L5        74 S L3 AND L4  
L6        29 DUP REM L5 (45 DUPLICATES REMOVED)  
L7        44795 S INCLUSION (A) BOD?  
L8        1 S L6 AND L7  
L9        42 S "LAMBDA PR"  
L10      0 S L2 AND L9  
L11      0 S L1 AND L9  
L12      0 S L9(W) LAMBDA PL  
L13      0 S "LAMBDA PR-LAMBDA PL"  
L14      35 DUP REM L9 (7 DUPLICATES REMOVED)

=> e kuppusamy m/au

E1        8        KUPPUSAMY KARTHIKEYAN/AU  
E2        1        KUPPUSAMY KAVITHA/AU  
E3        40 --> KUPPUSAMY M/AU  
E4        2        KUPPUSAMY M R/AU  
E5        1        KUPPUSAMY MUSAVAN/AU  
E6        2        KUPPUSAMY N/AU  
E7        2        KUPPUSAMY NALLAGOUNDER/AU  
E8        447      KUPPUSAMY P/AU  
E9        1        KUPPUSAMY PARIANNAN/AU  
E10      1        KUPPUSAMY PERIANNAM/AU  
E11      244      KUPPUSAMY PERIANNAN/AU  
E12      1        KUPPUSAMY R/AU

=> s e3-e6

L15      45 ("KUPPUSAMY M"/AU OR "KUPPUSAMY M R"/AU OR "KUPPUSAMY MUSAVAN"/AU OR "KUPPUSAMY N"/AU)

=> e mosuvan m/au

E1        1        MOSUSU N/AU  
E2        1        MOSUTO AGATIELLO L/AU  
E3        0 --> MOSUVAN M/AU  
E4        12      MOSUWAN L/AU  
E5        5        MOSUWE E/AU  
E6        1        MOSUWUN L/AU  
E7        1        MOSVASSAGHI A/AU  
E8        1        MOSVICHEV I F/AU  
E9        1        MOSVICK J/AU

E10 80 MOSVOLD J/AU  
E11 1 MOSVOLD JACOB/AU  
E12 1 MOSVSICHOFF F/AU

=> e mosuvan k/au  
E1 1 MOSUSU N/AU  
E2 1 MOSUTO AGATIELLO L/AU  
E3 0 --> MOSUVAN K/AU  
E4 12 MOSUWAN L/AU  
E5 5 MOSUWE E/AU  
E6 1 MOSUWUN L/AU  
E7 1 MOSVASSAGHI A/AU  
E8 1 MOSVICHEV I F/AU  
E9 1 MOSVICK J/AU  
E10 80 MOSVOLD J/AU  
E11 1 MOSVOLD JACOB/AU  
E12 1 MOSVSICHOFF F/AU

=> e vellimedu s/au  
E1 1 VELLIKY R S/AU  
E2 1 VELLILA P/AU  
E3 0 --> VELLIMEDU S/AU  
E4 11 VELLIMEDU S K/AU  
E5 6 VELLIMEDU SRINIVAS K/AU  
E6 1 VELLIN F/AU  
E7 1 VELLIN J F/AU  
E8 4 VELLIN P/AU  
E9 1 VELLINE C O/AU  
E10 1 VELLINES JOHN B/AU  
E11 1 VELLINES R P/AU  
E12 2 VELLING A/AU

=> e lahirি s/au  
E1 6 LAHIRI RANGAN/AU  
E2 4 LAHIRI ROMA/AU  
E3 1594 --> LAHIRI S/AU  
E4 2 LAHIRI S \*/AU  
E5 8 LAHIRI S A/AU  
E6 16 LAHIRI S B/AU  
E7 563 LAHIRI S C/AU  
E8 1 LAHIRI S C \*/AU  
E9 1 LAHIRI S C JR/AU  
E10 17 LAHIRI S D/AU  
E11 1 LAHIRI S H/AU  
E12 257 LAHIRI S K/AU

=> s e3  
L16 1594 "LAHIRI S"/AU

=> e krishna e/au  
E1 1 KRISHNA DUPPALAPUDI MURALI/AU  
E2 1 KRISHNA DUTTA S/AU  
E3 0 --> KRISHNA E/AU  
E4 10 KRISHNA E M/AU  
E5 1 KRISHNA E M GOPALA/AU  
E6 6 KRISHNA E MURALI/AU  
E7 1 KRISHNA E V/AU  
E8 1 KRISHNA EN R/AU  
E9 1278 KRISHNA G/AU  
E10 4 KRISHNA G \*/AU  
E11 20 KRISHNA G A/AU  
E12 2 KRISHNA G AMAR/AU

=> s e3-e7

L17        18 ("KRISHNA E"/AU OR "KRISHNA E M"/AU OR "KRISHNA E M GOPALA"/AU  
OR "KRISHNA E MURALI"/AU OR "KRISHNA E V"/AU)

=> d his

(FILE 'HOME' ENTERED AT 09:47:34 ON 11 MAY 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,  
LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004

L1        40597 S STREPTOKINASE?  
L2        362 S "S. EQUISIMILIS"  
L3        103 S L1 AND L2  
L4        6513995 S CLON? OR EXPRESS? OR RECOMBINANT  
L5        74 S L3 AND L4  
L6        29 DUP REM L5 (45 DUPLICATES REMOVED)  
L7        44795 S INCLUSION (A) BOD?  
L8        1 S L6 AND L7  
L9        42 S "LAMBDAPR"  
L10      0 S L2 AND L9  
L11      0 S L1 AND L9  
L12      0 S L9(W) LAMBDAPL  
L13      0 S "LAMBDAPR-LAMBDAPL"  
L14      35 DUP REM L9 (7 DUPLICATES REMOVED)  
          E KUPPUSAMY M/AU  
L15      45 S E3-E6  
          E MOSUVAN M/AU  
          E MOSUVAN K/AU  
          E VELLIMEDU S/AU  
          E LAHIRI S/AU  
L16      1594 S E3  
          E KRISHNA E/AU  
L17      18 S E3-E7

=> s l16 or l17

L18      1612 L16 OR L17

=> s l1 and l18

L19      0 L1 AND L18

=> d his

(FILE 'HOME' ENTERED AT 09:47:34 ON 11 MAY 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,  
LIFESCI' ENTERED AT 09:47:55 ON 11 MAY 2004

L1        40597 S STREPTOKINASE?  
L2        362 S "S. EQUISIMILIS"  
L3        103 S L1 AND L2  
L4        6513995 S CLON? OR EXPRESS? OR RECOMBINANT  
L5        74 S L3 AND L4  
L6        29 DUP REM L5 (45 DUPLICATES REMOVED)  
L7        44795 S INCLUSION (A) BOD?  
L8        1 S L6 AND L7  
L9        42 S "LAMBDAPR"  
L10      0 S L2 AND L9  
L11      0 S L1 AND L9  
L12      0 S L9(W) LAMBDAPL  
L13      0 S "LAMBDAPR-LAMBDAPL"  
L14      35 DUP REM L9 (7 DUPLICATES REMOVED)  
          E KUPPUSAMY M/AU  
L15      45 S E3-E6  
          E MOSUVAN M/AU  
          E MOSUVAN K/AU  
          E VELLIMEDU S/AU

E LAHIRI S/AU  
L16 1594 S E3  
E KRISHNA E/AU  
L17 18 S E3-E7  
L18 1612 S L16 OR L17  
L19 0 S L1 AND L18